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Behavioral Testing as a Method for Assessing Risk

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Behavioral effects have been found to result from the prenatal administration of substances known to be teratogenic to the CNS. These effects occur at dose levels lower than those producing gross malformations and when the agent is administered at times other than that optimal for CNS teratogenesis. These findings have led to the belief that behavioral testing can be a sensitive and relevant technique for detecting adverse consequences of prenatal exposure to drugs and chemicals. Behavioral testing, however, also appears to have attributes that dictate a thoughtful approach to its role as a method for assessing risk, and additional research is needed to obtain usable techniques. The need for such research is intensified by the present inability to identify potential behavioral teratogens by means other than laboratory investigation.

Information about the behvioral effects of drugs administered during development is now required by the British and Japanese governments as part of their reproduction testing procedures. The United States guidelines also contain an optional provision calling for such behavioral testing, but to the best of my knowledge this option has never been exercised. These government actions have focused attention upon behavioral testing and its role as a deveice for assessing the undersirable consequences of environmental stress during development. Within this context, I would like to review a sample of the literature in this area, to offer what appear to be the implications of this research, and finally to offer some comments on behavioral testing as a tool for assessing risk.

I will limit my remarks to those circumstances usually denoted by the term "behavioral teratology;" that is, a set of circumstances in which an organism is exposed to a test agent at some time during its development and the consequences of that exposure is looked for in the behavior of the subject at a later time when the immediate effects of the agent, if any, have passed. In the usual procedure, the agent is administered during gestation, and the test subject is examined postnatally to determine if there are enduring effects. The six examples of research I have chosen are all of this type and although each undoubtedly has some faults, I believe they are a reasonably representative sample of experiments in this area. In each study (Table 1) the agent, vitamin A, was administered during gestation to the rat and behavioral tests were administered postnatally. Hypervitaminosis A is known to have a teratogenic effect, and, in addition, has been demonstrated to malform the CNS. The immediate impression made by these studies is one of great diversity—a wide variety of dose levels, times of administration, strains, and behavioral tests have been used. Perhaps this diversity has some importance, for at least we know that the phenomenon under study is rather general and is not strain specific or task specific.

Closer scrutiny of these experiments, however, reveals a common logic in the apparently diverse procedures. That logical thread is an attempt to

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Table 1. Summary of behavioral studies on the subteratogenic effect of hypervitaminosis A.

Author	Vitamin A dose, IU/kg/day	Time of administration, day(s)[a]	Strain	Physical effect	Tests	Result
Malakhovskii (*1*)	60,000 300,000	9	Albino rat	No report	Shock avoidance escape	*Impaired learning*
Malakhovskii (*2*)	150,000	9	Albino rat	No gross No microscopic	Activity Shock avoidance escape aggression	Hypoactive Impaired Reduced
Butcher (*3*)	100,000	8, 9, 10	Sprague-Dawley	+2% in gross *malformations*	Swimming *maze*	Reduced learning
Hutchings (*4*)	180,00	13, 14	Wistar	No gross Growth retardation Small brains	Discriminative operant	Poorer discrimination
Hutchings (*5*)	270,000	16, 17	Wistar	No gross No microscopic	Discriminative operant	Lower response rates
Vorhees (*6*)	100,000 40,000 25,000 10,000	8, 9, 10	Fischer 344	Growth retardation in 100,000 IU level	Y-maze Shock avoidance Activity	Impaired avoidance No difference

[a] Date of sperm = day 0.

detect a behavioral deficit in the absence of anatomic deformities. In almost every case the investigator(s) have offered some evidence indicating that anatomic defects are either absent or that the incidence of such malformations is low. In the Hutchings (*4,5*) studies an attempt has been made to demonstrate behavioral impairments resulting from exposure at a time during gestation when anatomic malformations are rare. incidentally, not only do these effects appear in the absence of malformation, but the effects of exposure on days 13-14 are different from those resulting from exposure on days 16-17.

On taking these studies in combination, what do we know about the effect of prenatal exposure to large amounts of vitamin A that we would not have known had the behavioral studies not been done? First, it would appear that hypervitaminosis A has an effect at doses lower than those that produce noteworthy anatomic defects. Stated in other terms, hypervitaminosis A has a functional (in this case behavioral) effect that precedes anatomic defects on the dose/response curve. In addition, the vitamin has an effect when administered at a time later in gestation, when the rat has been found to be more resistant to gross malformation. Neither of these findings is revolutionary—it would be unusual that all effects of an agent would end abruptly with the disappearance of gross anatomic effect, and it would be equally surprising if the CNS which differentiates over a long period would be vulnerable for just a few days during gestation.

From a more general perspective, what appear to be the implications from studies such as these for the role of behavioral testing as a tool in assessment of risk? The discovery of behavioral effects in the absence of morphological alteration of the CNS implies that behavioral testing is a sensitive technique for the detection of adverse consequences of prenatal environmental stresses. Where minor or infrequent anatomic abnormalities are found, alterations in behavior provide a demonstration of the functional significance of what might otherwise be regarded as anatomic "variants" having no functional consequences. The temporal relationship between the administration of the agent and the testing procedures also suggests an enduring effect.

So we may claim for the psychological test procedures two attributes not possessed in these instances by morphological examination, sensitivity, and relevance. It is, I believe, the quality of sensitivity that has provided the primary impetus for behavioral testing and some comments on the way in which this sensitivity is developed methodologically could be informative. To generalize again, it appears that behavioral tests are usually procedures that assay the performance of a test subject in situations requiring the use and integration of several primary functional systems. The overall ability of the subject to adapt to the experimental situation is what is judged, and deficits in any subsystem that contribute to the overall performance become apparent. Such a test is often referred to as apical, a single test re-

quiring the successful integration of intact subsystems. The performance of even a simple maze task, for example, involves the interplay of motivational, sensory, learning, and motor capacities—and this is the most gross level of analysis possible.

Under conditions of this type of apical testing, it is less surprising that behavioral testing is a sensitive evaluation technique because the tasks summarize in a single measure of performance the contributions of a number of systems that may have been harmed by the test agent. The psychological testing process is also a nondestructive technique and such summary performance can not only be tested once, but again and again. To go even further along these lines, you will notice that in many such tests each successive step is contingent upon the preceding one, and that flaws in performance are carried forward.

So there appears to be some logical basis for the sensitivity of behavioral testing which is inherent in the methods. The sensitivity of these methods, however, does not come without cost for just as a particular weakness in a system will be reflected in a reduction in overall performance, so can particular strengths compensate. As Rodier (7) has correctly pointed out in her review, behavioral testing can illustrate the subjects ability to perform despite injury. It is a continual source of wonder how much as animal can do with the little bit of brain tissue left to it after surgery, and the literature of psychology is replete with examples of compensation for brain injury. Just what sorts of injury are likely to escape detection despite the sensitivity of the behavioral test and what kinds of damage are particularly likely to be revealed is not known. This appears to be an area toward which some research might profitably be directed. In any case, the sensitivity of the behavioral test is a one-way judgement, the results can indict, but not acquit. Deficient performance means "something is wrong," but adequate performance does not mean "nothing is wrong."

Unfortunately for the investigator, the behavioral performance of a subject is also a sensitive indicator of influences other than the test agent under consideration. The number of these influences form an intimidating list of variables to be controlled. All aspects of selecting, housing, and handling of the dam including those surrounding the administration of the agent, the housing, handling, fostering, size, and sexual composition of the litter must be carefully controlled before the first subject is examined. Testing then brings with it another long list of influences that can intrude upon the detection of a treatment effect. The necessity of controlling these numerous influences over a considerable length of time is a time consuming and expensive proposition when compared to the techniques presently used in reproductive testing.

These characteristics of behavioral testing have implications for the way a psychological evaluation might be used in the assessment of risk process. For example, until more efficient behavioral techniques in this area are developed, it appears likely that behavioral testing would be among the final tests administered. Thus, the apical tests would be used in the apical situation—after something is known about the morphological and physiological effects of the agent studied—and the question asked, "has something been missed?" A benefit of using a psychological test series in later stages would be the possibility of examining the effects of an agent when administered in an amount closer to the anticipated therapeutic dose. The sensitivity of the behavioral test could be exploited in a way that would provide direct information about moderate exposure levels and a more realistic estimate of the range of response than would be provided by extrapolations from teratologic studies.

Examining the functional capacity of the test subject would also engage a new and compelling set of health problems. Functional deficits and behavioral defects in particular constitute a chronic public health problem, and insofar as we can imagine that they may result from a prenatal insult, we should endeavor to identify and eliminate these insults. If we allow the possibility of a behavioral impairment from prenatal exposure to an environmental agent, we must also consider the possibility of detecting that effect. Under present circumstances our ability to identify the relationship between a prenatal stress and its effect on behavior is extraordinarily limited. Indeed, almost all human teratogenic effects have been identified clinically rather than in the laboratory. Many of you are aware of the difficulty with which thalidomide was identified as a teratogen under circumstances in which the abnormality produced was gross, rather unique, and obvious at birth. Consider, in light of that history, how small would be the probability of detecting the cause-and-effect relationships between an agent administered during pregnancy and an impairment in learning ability. Such an impairment would not be an uncommon event, would represent a rather subtle effect, and would be diagnosed almost certainly after the fourth year of

postnatal life. It would seem that we must place increased reliance upon laboratory investigation, because our ability to identify possible behavioral teratogens is so very limited.

I have tried in this brief review to provide a very general perspective on behavioral testing as it might be used in reproductive studies designed to assess risk. Such investigations can have benefits and I have tried to point some of the cautions that seem appropriate. If such testing is to be used, however, we must very rapidly become specific about the design, method and interpretation of such studies. This sort of specific information is not available and it is going to take some hard work to develop usable, meaningful tests. The final comment must be a call for research.

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